REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. CLAIM STATUS & AMENDMENTS

Claims 23-26 and 29 (now 27) were erroneously presented as the pending claims in the response filed November 7, 2003. The present amendment correctly renumbers claim 29 as claim 27. Thus, claims 23-27 were pending and rejected in this application when last examined.

Claim 23 has been amended to recite in the preamble that the drug tolerance is at least 1,000 times higher than that of wild type *Escherichia coli*. Support can be found in the specification, for example, at Table 1 on page 21, and Tables 3-5 on pages 24-26.

Claim 23 has also been amended to make minor grammatical changes.

Therefore, no new matter has been added by this amendment.

Claims 23-27 are pending in this application.

II. CLAIM OBJECTIONS

Claim 29 is objected to on the basis that it was erroneously numbered claim 29 in the response filed November 7, 2003. See Office Action, page 2, 3rd paragraph. The present amendment correctly renumbers this claim, thereby obviating this objection.

III. REJECTION UNDER 35 U.S.C. § 103(a)

Claims 23-27 are rejected under 35 U.S.C. § 103(a) as obvious over Fijalkowska et al. and Lin et al. in view of either Imamoto et al. or Iwaki et al. and further in view of Pan et al. See Office Action, pages 3-6.

This rejection is respectfully traversed as applied to the amended claims for the same reasons set forth in the response filed November 7, 2003 and for the following reasons.

Claim 23 is directed to a novel method for establishing a mutant of *Escherichia coli* dnaQ49 strain having a tolerance to an antibiotic drug which is at least 1,000 times higher than that of the wild type *Escherichia coli*. Step (a) of claim 23 is directed to a method for introducing a mutation into the genomic of *Escherichia coli* dnaQ49 strain by culturing it under a certain temperature. In step (b) of claim 23, a mutant *Escherichia coli* dnaQ49 strain, which is tolerant to the drug, is then selected. Step (c) of claim 23 calls for repeating steps (a) and (b) to develop increased tolerance of the mutant to the drug until the tolerance is at least 1,000 times higher than the wild type tolerance. Moreover, step (b) is repeated under a higher concentration drug than in the prior step (b), and step (a) is repeated under the same concentration of the drug as in step (b) immediately therebefore. A detailed description of the claimed method can be found on pages 18-20 of the specification.

The most important aspect of the claimed invention is producing mutants of *Escherichia coli* dnaQ49 strain having ***tolerance to antibiotic drugs at least 1,000 times higher than wild type***. That is, mutant dnaQ strains capable of growing in the presence of 6,000 μg/ml of ampicillin, of 500 μg/ml of ofloxacin, 7,000 μg/ml of nalidixic acid, and of 26,000 μg/ml of streptomycin as disclosed in the specification, for example, at Table 1 on page 21, and Tables 3-5 on pages 24-26.

As argued in the prior response, the cited references fail to render obvious the claimed invention, because they fail to teach or suggest a method of obtaining such an *Escherichia coli* dnaQ49 strain having a 1,000-fold increase in tolerance over the wild type. In the last lines on page 5 of the Office Action, it is acknowledged that the cited references fail to disclose the specific level of increased tolerance. Despite this deficiency, the Office argues that Pan discloses the repetition of steps of selection under increasing conditions of antibiotics, and thus, the specific level of 1,000 fold increase in tolerance would have been achieved by routine experimentation. See Office Action, paragraph bridging pages 5-6.

Applicants respectfully disagree with the position that Pan suggests the necessary repetition of the steps of selection to attain the specific level of 1,000-fold increase in tolerance. No where in Pan or the cited references is it disclosed or suggested that the repetition of the steps of selection will produce *Escherichia coli* having tolerance to antibiotic drugs on the scale of at least 1,000 times

higher than wild type. Pan also fails to even teach a mutagenesis step using a mutator gene. Thus, Pan cannot be said to teach introducing a mutation using such a method.

Moreover, the cited references ***lack a reasonable expectation of success*** for achieving the specific level of increased tolerance. In this regard, no evidence existed at the time of the claimed invention for the existence of *Escherichia coli* strain having drug tolerance 1,000-fold higher than wild type. In fact, the highest resistance concentration of ampicillin-resistant *Escherichia coli* which had been reported at the time of this invention was 1,500 μg/ml, i.e. a tolerance of only 250-500 times the wild type of 3-6 μg/ml and the resistance was due to a plasmid. When mutagenesis was carried out at 37°C without addition of ampicillin, it was not possible to obtain an ampicillin-resistant microorganism even by ten operations. As a result of the claimed method, resistant microorganisms showing a resistance to ampicillin of unexpectedly high concentrations were able to be acquired within a short period.

Based on such an understanding of the nature of the art, one of ordinary skill in the art at the time of the claimed invention would not reasonably have expected to use the prior art teachings to attain a mutant with a 1,000 fold increase in tolerance. Even if the *Escherichia coli* dnaQ strain was known to be a temperature-sensitive mutant that increases the frequency of mutation, it was not known that such a mutant strain could exhibit drug tolerance 1,000-fold higher than wild type. None of the cited references disclose or suggest such.

Furthermore, as argued in the prior response, the cited references also ***fail to disclose each and every element of the of the repetition method in step (c)*** of claim 23.

Claim 23 calls for repeating steps (a) and (b) to develop increased tolerance of the mutant to the drug until the tolerance is at least 1,000 times higher than the wild type tolerance. Moreover, step (b) of claim 23 is repeated under a higher concentration drug than in the prior step (b), and step (a) is repeated under the same concentration of the drug as in step (b) immediately therebefore. Contrary to the position on page 5, lines 3-18 of the Office Action, none the cited references disclose or suggest this claimed feature.

Pan is relied upon as allegedly disclosing steps in which the increased selection load was not identical to that of the previous step, and other steps in which the selection load was the same as that of the previous step. See Office Action, pages 3-4 and 5. However, such a teaching fails to suggest a method for repeating step (b) under a higher concentration drug than in the prior step (b), and repeating step (a) under the same concentration of the drug as in step (b) immediately before. Also, Pan fails to teach the step of mutagenesis using a mutator gene and specific conditions for the mutator gene, such as temperature-sensitivity.

As acknowledged on page 3 of the Office Action, the remaining references of Fijalkowska, Lin, Imamoto and Iwaki fail to rectify this deficiency. None of these references disclose a selection load nor the specifically claimed method steps.

In summary, the cited references lack a reasonable expectation of success, they fail to teach or suggest each and every element of the claimed invention, and it would take more than routine experimentation to arrive at the claimed invention. Therefore, it is respectfully submitted that the method of claim 23 could not have been expected from the combined teachings of the prior art. In addition, the mutants of claims 24-27 could not have been expected.

CONCLUSION

In view of the foregoing amendments and remarks, the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Favorable reconsideration and allowance is thus respectfully solicited.

Respectfully submitted,

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September 27, 2004